

Abstract No. Crai0179

Solving Crystal Structures of a Trypanosomal Hypoxanthine Phosphoribosyltransferase with Lead Inhibitors Bound in the Active Site

A. Virani, N. Silvaggi, J. Kelly, and S. Craig

Beamline(s): X12B

Introduction: *Trypanosoma cruzi* is the etiologic agent of Chagas' disease, which afflicts 16-18 million people in Latin America and more than 300,000 immigrants to the United States. Presently, there are no drugs available for the treatment of chronic Chagas' disease and new drugs are urgently needed. The long-range goal of our research program is to rationally design novel inhibitors of a target enzyme (hypoxanthine phosphoribosyltransferase or HPRT) in order to develop prospective drugs. Our starting point has been the crystal structure of the trypanosomal HPRT in a closed conformation [1]. This well-defined structure enabled the computational identification of lead compounds which were potent inhibitors of the target enzyme and showed biological activity against the pathogen [2].

Methods and Materials: A variety of conditions for protein crystallization have been tested, both with and without lead inhibitors dissolved in the hanging drops. Subtle modifications of the conditions for which three-dimensional crystals grow have been tested in our efforts to generate better diffracting crystals. Micro-seeding and soaking strategies also have been employed to generate crystals with specific inhibitors bound in the active site of the target enzyme. Data sets collected at NSLS have been solved by methods of molecular replacement and the crystal structures will be used as guides for organic synthetic strategies intended to generate novel compounds with enhanced affinity and specificity for the target enzyme, and with improved pharmacological properties.

Results: The X12B beamline of the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratory has been and continues to be a valuable resource in that we have been able to collect high-resolution data sets of the trypanosomal HPRT with various ligands bound in the active site. Structures solved at the NSLS are reported in a manuscript submitted for publication [3].

Conclusions: We continue in our efforts to solve structures of the trypanosomal HPRT with lead inhibitors bound in the active site in order to optimize the structures of the drug leads.

Acknowledgments: This work was supported by National Institutes of Health Grant Numbers AI-38919 and AI-45021. National Synchrotron Light Source (NSLS) is supported by the Department of Energy, Division of Materials Sciences and the Division of Chemical Sciences.

References:

- [1]. P.J. Focia, S.P. Craig III and A.E. Eakin, "Approaching the transition state in the crystal structure of a phosphoribosyltransferase," *Biochemistry* **37**, 17120-17127, 1998.
- [2]. D.M. Freymann, M.A. Wenck, J.C. Engel, J. Feng, P.J. Focia, A.E. Eakin, and S.P. Craig III, "Efficient identification of inhibitors targeting the closed active site conformation of the HPRT from *Trypanosoma cruzi*," *Chem. & Biol.* **7**, 957-968, 2000.
- [3]. B. Canyuk, F.J. Medrano, M.A. Wenck, P.J. Focia, A.E. Eakin and S.P. Craig III, "Interactions at the Dimer Interface Influence the Relative Efficiencies for Purine Salvage and Pyrophosphorolysis in a Phosphoribosyltransferase," submitted: *J. Biol. Chem.*, 2002.